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Final Summary Report

"Chemical Methods for the Production of Proteins."
Stephen Kent, P.I.

The goal of this research program was to develop improved methods for chemical peptide and protein synthesis, and to apply these methods to the total synthesis of small proteins (<80 amino acids) & integral membrane proteins.

Over the four years of this research program, several novel chemical protein synthesis methods were developed:

- 'One pot' native chemical ligation, for the facilitated synthesis of small proteins
- His_6-tag assisted chemical protein synthesis
- An improved catalyst for native chemical ligation, 'MPAA'
- 'Kinetically controlled ligation', for the fully convergent chemical synthesis of larger proteins
- 'Solid phase chemical ligation' for the polymer-supported synthesis of small protein molecules
- Selecitve desulfurization of Cys in the presence of Cys(Acm)

These improved chemical synthesis methods were prototyped on a series of chemical protein syntheses that included:

- total synthesis of protein diastereomers (D-amino acid containing L-proteins)
- elucidation of the energetics of C- helix capping in ubiquitin
- a photocleavable analog of yeast alpha-factor
- total synthesis and high resolution Xray structures of enzymes
- potassium channel accessory protein

The novel methods and resulting syntheses are described in a series of published scientific papers acknowledging support from the DOE GTL-Genomics research program

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Publications

Twenty (20) primary refereed publications in top-tier scientific journals, including two journal covers, resulted from this research program (see Appendix).

Intellectual Property

One comprehensive patent filing resulted from this research program (see Appendix). Support by the DOE was acknowledged.

Primary refereed papers published:

1. Total chemical synthesis of Crambin. D. Bang, N. Chopra, S.B.H. Kent, *J. Am. Chem. Soc.,* **126**, 1377-83 (2004)

2. A one-pot chemical synthesis of Crambin. D. Bang, S.B.H. Kent, *Angew. Chem. Int. Ed. Eng.,* **116**, 2588-2592 (2004)

3. His_6 tag-assisted chemical protein synthesis. D. Bang, S.B.H. Kent, *Proc. Nat. Acad. Sci. USA*, **102**, 5014-5019 (2005)

4. Total chemical synthesis and X-ray crystal structure of a protein diastereomer: [D-Gln35]Ubiquitin. Duhee Bang, George I. Makhatadze, Valentina Tereshko, Anthony A. Kossiakoff, Stephen B. Kent, *Angew. Chem. Int. Ed. Eng.,* **44**, 3852-3856 (2005)

5. [Cover article]Dissecting the energetics of protein α-helix C-cap termination through chemical protein synthesis. Duhee Bang, Alexey V. Gribenko, Valentina Tereshko, Anthony A. Kossiakoff, Stephen B. Kent*, George I. Makhatadze*, *Nature Chemical Biology*, **2**, 139-43 (2006)

6. Direct on-resin synthesis of peptide-αthiophenylesters for use in native chemical ligation. Duhee Bang, Brad L. Pentelute, Zachary P. Gates, and Stephen B. Kent, *Organic Letters*, **8**, 1049-52 (2006)

7. Insights into the mechanism and catalysis of the native chemical ligation reaction. Erik C.B. Johnson, Stephen B.H. Kent, *J. Am. Chem. Soc.,* **128**, 6640-6 (2006)

8. Studies on the insolubility of a transmembrane peptide from Signal Peptide Peptidase. Erik C.B. Johnson, Stephen B.H. Kent, *J. Am. Chem. Soc.,* **128**, 7140-1 (2006).

9. Synthesis, stability and optimized photolytic cleavage of 4-methoxy-2-nitrobenzyl backbone-protected peptides. Erik C.B. Johnson and Stephen B.H. Kent, *Chemical Communications*, 1557-9 (2006).

10. [Cover article]Kinetically-controlled ligation for the convergent chemical synthesis of proteins. Duhee Bang, Brad Pentelute, Stephen B.H. Kent, *Angew Chem Int Ed Engl*., **45**, 3985-3988 (2006).

11. Total chemical synthesis, folding, and assay of a small protein on a water-compatible solid support. Erik C.B. Johnson, Thomas Durek, Stephen B.H. Kent, *Angew Chem Int Ed Engl.,* **45**, 3283-7 (2006).

12. Control of the yeast cell cycle with a photocleavable α-factor analog.

Laurie L. Parker, Stephen B. H. Kent, Stephen J. Kron, *Angew Chem Int Ed Engl*, **45**, 6322-5 (2006).

13. Towards the total chemical synthesis of integral membrane proteins: a general method for the synthesis of hydrophobic peptide-$^{\alpha}$thioester building blocks. Erik C.B. Johnson, Stephen B.H. Kent, *Tetrahedron Letters*, **48**, 1795-99 (2007).

14. An exploratory synthesis of peptide-αthioester segments spanning the polypeptide sequence of the δ-Opioid Receptor, a G protein-coupled receptor. Erik C.B. Johnson, Stephen B.H. Kent, *Biopolymers: Peptide Science*, **88**, 340-9. (2007).

15. Convergent chemical synthesis and crystal structure of a 203 amino acid 'covalent dimer' HIV-1 protease enzyme molecule. Vladimir Yu. Torbeev, Stephen B. H. Kent, *Angew Chem Int Ed Eng*, **46**, 1667-70 (2007).

16. Selective desulfurization of cysteine in the presence of Cys(Acm) in polypeptides obtained by native chemical ligation. Brad Pentelute, Stephen Kent, *Organic Letters*, **9**, 687-90 (2007).

17. Convergent chemical synthesis and high resolution X-ray structure of human lysozyme. Thomas Durek, Vladimir Yu. Torbeev, Stephen B. H. Kent, *Proc. Natl. Acad. Sci. USA,* **104**, 4846-4851 (2007).

18. Insights from atomic-resolution X-ray structures of chemically synthesized HIV-1 protease in complex with inhibitors. Erik C.B. Johnson, Enrico Malito, Yuequan Shen, Brad Pentelute, Dan Rich, Jan Florián, Wei-Jen Tang, Stephen B.H. Kent. *J Molecular Biol,* **373**, 573-86 (2007).

19. Modular total chemical synthesis of an HIV-1 protease. Erik C.B. Johnson, Enrico Malito, Yuequan Shen, Dan Rich, Wei-Jen Tang, Stephen B.H. Kent, *J Am Chem Soc*, **129**, 11480-90 (2007).

20. Total chemical synthesis and biophysical characterization of the minimal isoform of the KChIP2 potassium channel regulatory subunit**.** Sudarshan Rajagopal, Stephen B.H. Kent, *Protein Sci*, **16**, 2056-64 (2007).

U.S. Patent Application No. 11/545,923 "Convergent Synthesis of Proteins by Kinetically Controlled Ligation"

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